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(54) Title: MICROPOROUS POLYMER MATRICES

(57) Abstract: A method for the manufacture of a microporous polymer matrix comprises the steps of: a) introducing into a mould a solution of polymer in a solvent, and b) adding a precipitant to the solution so as to cause precipitation of the polymer from the solution in a form which retains the shape of the mould upon removal from the mould. Useful polymers for carrying out the method of the invention are those which show a tendency to crystallise from solution on solvent extraction. These include poly(L-lactide) or copolymers of poly(L-lactide) and a poly (α-hydroxy acid) which will dissolve in acetone and subsequently crystallise upon solvent extraction. The preferred polymer is poly(ε-caprolactone). The precipitant is preferably a solvent in which the polymer is substantially insoluble. Preferred precipitants are polar solvents such as alcohols, in particular methanol.

Title: Microporous Polymer Matrices

This invention relates to the manufacture and use of microporous polymer matrices. Such materials may be useful for formulating controlled release devices for bioactive molecules such as growth factors and hormones and for fabricating scaffolds to support cell growth and development in tissue engineering.

Poly(ε-caprolactone) (PCL) is a synthetic α-polyester exhibiting a low Tg of around -60°C which imparts a rubber characteristic to the material. PCL like other members of this family of polymers such as poly(L.lactide) (PLA) and poly(lactide co-glycolide) (PLG), undergoes auto-catalysed bulk hydrolysis but the semi-crystalline nature of the polymer extends its resorption time to over 2 years. The rubbery characteristics of the polymer results in high permeability which has been exploited for delivery of low molecular weight drugs such as steroids. Copolymerisation of lactic acid and ε-caprolactone has been investigated to increase degradation rates and improve processability. However the poor mechanical properties of the copolymers have limited the number of applications for these copolymers.

Tissue engineering combines the biological, physical and chemical sciences with engineering disciplines to design and manufacture implants for repair, support, augmentation or replacement of damaged or diseased tissues and organs such as bone and skin. In one approach, bioactive molecules such as growth factors (eg bone morphogenetic protein (BMP), vascular endothelial growth factor (VEGF)) are released in a controlled fashion from an implanted device to modulate processes of cell interaction and adhesion, cell proliferation and cytodifferentiation so as to effect tissue repair or regeneration. In a second approach, cells are first incorporated into 3-D scaffolds or matrices ex-vivo. The cell-scaffold construct is subsequently transplanted and cell development continues to bring about repair and regeneration of tissues such as cartilage.

The microstructure and architecture of the scaffold exerts profound effects on cell alignment through contact guidance effects and governs the extent of tissue

ingrowth and overgrowth which subsequently determines the quality of integration of new and host tissue. Scaffold design has been found to influence cell distribution, orientation and viability. The void volume and pore size also influences extra cellular matrix formation (Zeltinger et al, Advances in Tissue Engineering and Biomaterials, 1st Smith and Nephew Symposium, York 1997). Designing the scaffold to mimic the cell environment in the host tissue could be advantageous for achieving an appropriate cell arrangement and density so as to induce correct cell differentiation and function. Macropores increase the surface area for cell attachment and permit tissue ingrowth. In addition, the scaffold material should be microporous to allow exchange of nutrients and metabolites and may also provide a favourable surface topography for cell attachment.

Implant porosity can influence tissue repair by allowing limited tissue ingrowth for stabilisation of permanent implants or by providing pathways for tissue regeneration within or over a biological scaffold or matrix. The pore size and structure of interconnections will determine the extent of tissue ingrowth. A minimum pore size of approximately 100μm in bioceramics has been defined for effective bone ingrowth (Klawitter, J.J. and Hulbert, S.P., J. Biomed. Mater. Res. 2 (1971), 161). The optimum pore size is sufficient to accommodate blood vessel formation with closely associated osteocytes. The pore size in synthetic polymer implants is also known to be a critical factor in determining the prevalence of fibrous or bony tissue generation. Bony ingrowth predominated in porous polymethylmethacrylate (PMMA) when the pore size was around 450μm, whereas connective tissue formed in PMMA implants having a pore size less than 100μm (Ashman, A., Moss, M.L., J. Prosthet. Dentistry, 37 (1977) 657-65). Extensive vascular infiltration was obtained with a pore size of 1000μm. In the case of bone implants, therefore, inappropriate pore size and structure can stimulate an undesirable fibrous tissue response. A structure comprising macropores (150-300μm) highly interconnected by micropores (smaller than 50μm) was found to be conducive to ingrowth of fibrocartoilaginous tissue in polyurethane (PU) meniscal implants (deGroot et al, Biomaterials, 17 (1996) 163-173).

The scaffolds for seeded cells may be fabricated by a variety of techniques and from a wide range of materials, both natural (eg collagen, alginate) and synthetic (eg resorbable polyglycolic acid (PGA) and polydioxanone). One of the main advantages of synthetic materials is that physicochemical characteristics such as strength, stiffness, degradation rate and microstructure can be controlled during manufacture. Natural polymers present advantages of biocompatibility and can promote cell adhesion to the supporting scaffold.

A large number of fabrication techniques have been investigated for production of blocks of porous, polymeric, implant materials (Coombes, A.G.A., Meikle, M.C. Clinical Materials, 17 (1994) 35-67). These include packing of PLG precipitates in a mould followed by heating, gel casting using PLA or PLG solutions followed by solvent extraction and drying, variation of the proportion of lactide polymer and particulate fillers to provide voids or pores in the material, extraction of low molecular weight additives such as sodium citrate from the solid polymer and machining of macropores in preformed blocks of material.

Micro/macroporous PU materials have been produced by a freeze drying/salt leaching process. Salt crystals were mixed with a pre-polymer/monomer solution and freeze dried at -15°C. Removal of the solvent by sublimation produced the microporous structure (<50µm). After curing the prepolymer/monomer/salt mixture, the salt was removed by washing to leave 150-300µm macropores.

Micro/macroporous PLA implants have also been produced by direct machining of 500µm channels in a block of microporous, gel-cast material (US 5,290,494).

Porous tubular materials have been described which were produced by spray or dip coating PGA, non-woven mesh (12µm diameter fibres) using solutions of PLA or PLG (Mooney et al, Biomaterials 17 (1996) 115-124).

Porous biodegradable PLG scaffolds for nerve regeneration have been produced by melt extrusion of a PLG/salt mixture (150-300µm crystals) to form a tube,

followed by salt leaching and vacuum drying (Brandt et al., 1st Smith & Nephew Conference, York, 1997).

Open porous PMMA materials (300-1500μm mean pore diameter) for use in spinal fusion were prepared by phase separation of a mixture of PMMA and aqueous polymer solution (Wintermantel et al, Biomaterials, 17 (1996) 83-91).

Scaffolds for tissue engineering are required to provide mechanical support of developing tissue and also structural and biochemical cues to guide and organise developing tissue. Complex scaffold architectures for bone regeneration have been produced by solid free-form fabrication in which thin layers of the scaffold are built up sequentially by a 3-D printing technique. Control over the size, orientation and material composition of pores and channels is possible to modulate tissue ingrowth. Patterned surface modifications have also been considered for spatial control of cell adhesion and migration (Koegler et al, Ist Smith & Nephew Conference, York, 1997).

Porosity is inherently present in fibrous materials such as fabrics, felts and mesh and as such these materials have been investigated for a variety of tissue engineered constructs. Knitted fabrics, for example, exhibit three types of porosity: 1) the open space within interlocked loops, 2) the inter-filament spaces, and 3) inter-layer spaces (Wintermantel et al, Biomaterials, 17 (1996) 83-91). Non-woven PGA mesh produced from 12μm diameter fibres and having an inherent porosity of 97% has been used extensively as a scaffolding material for seeding cells to produce tissue engineered constructs. Mechanical stabilisation of the PGA mesh has been achieved by spray coating or dip coating with a second polymer and by using thermal bonding techniques (Mikos et al, J. Biomed. Mater. Res. 27, (1993) 183).

Blending of polymers to achieve a balance in material properties has been applied extensively in biomedical materials and drug delivery research. In the former area, microporous materials for replacing bone graft have been produced by blending fast resorbing PLG and slow resorbing PLA to match the resorption rate and

dimensional stability of the resulting material with local requirements of tissue repair.

PCL is much more permeable than PLG for delivery of bioactive materials but degrades very slowly. Blending of PCL and PLG has been shown to result in retention of permeability and form stability while increasing the overall degradation rate of the blend.

There has now been devised a method for the production of microporous polymer matrices, which offers significant advantages in the formulation of controlled release devices for bioactive molecules, in the fabrication of scaffolds for tissue engineering, or in other applications.

According to the invention, there is provided a method for the manufacture of a microporous polymer matrix, which method comprises the steps of

a) introducing into a mould a solution of polymer in a solvent, and

b) adding a precipitant to the solution so as to cause precipitation of the polymer from the solution in a form which retains the shape of the mould upon removal from the mould.

Step a) may be carried out by forming the solution in the mould, ie by adding the polymer and solvent separately to the mould. Alternatively, the solution may be made up externally and poured or otherwise introduced into the mould.

The solvent is preferably an organic solvent. Acetone is currently the most preferred solvent. Another suitable solvent is ethyl acetate. The suitability of a particular solvent for use in the method of the invention may be readily determined. In general, a solvent will be suitable if the polymer dissolves in it and is precipitated upon addition of the precipitant in a form which retains the shape of the mould.

The precipitant is preferably a solvent in which the polymer is substantially insoluble. Preferred precipitants are polar solvents such as alcohols, in particular methanol. The suitability of a particular material as a precipitant for use in the method of the invention may be readily determined. For instance, visual inspection of the effect of addition of a particular material may indicate whether it is effective in precipitating the polymer in a form suitable for use in the invention.

For successful performance of the invention it may be necessary for the concentration of polymer in the solvent to be above a certain threshold or critical level. Again, optimisation of the polymer concentration may be readily carried out.

In a particularly preferred embodiment of the invention, precipitation occurs initially at the solution/precipitant interface, thereby forming a semi-permeable polymer membrane across which the solvent is extracted. Solidification may then occur by gradual crystallisation along a front proceeding from the solution/precipitant interface. In such an embodiment the polymer solution and the precipitant form distinct layers when brought carefully into contact. This characteristic is potentially useful for producing directionally solidified materials which exhibit oriented textures or morphologies for improving cell contact guidance.

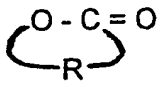
Precipitation of the polymer at the polymer solution/precipitant interface may be rapid, occurring on a timescale of the order of minutes. Complete solidification of the moulded matrix may be more protracted, taking hours or days to complete, depending on, for instance, the dimensions and geometry of the mould.

The solvent power should generally be such that polymer precipitation results from extraction of the solvent across the semi-permeable membrane formed at the solvent/precipitant interface. To achieve optimum performance, mixtures of solvents may be used. Such mixtures might include mixtures of a preferred solvent such as acetone with another solvent which may be less suitable when used on its own, eg dichloromethane.

The microporous materials obtained may be resilient and may be readily compressible. This facility offers the potential for varying the density and subsequently altering the drug release characteristics from these materials by controlled compression of drug-loaded matrices.

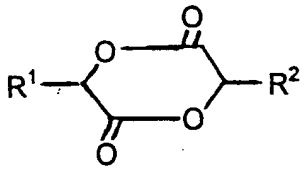
PCL is the preferred polymer. Useful polymers for carrying out the method of the invention are those which show a tendency to crystallise from solution on solvent extraction. These include poly(L.lactide) or copolymers of poly(L.lactide) and a poly (α-hydroxy acid) which will dissolve in acetone and subsequently crystallise upon solvent extraction.

Suitable polymers may thus be members of the class of polyesters formed by ring-opening polymerisation. Precursors to such polymers may thus have the generic formula



in which R represents an optionally substituted alkylene chain, eg a chain $(CH_2)_n$ in which n is an integer of from about 4 to 10. In the particularly preferred case of ε-polycaprolactone, n is 5.

Another specific group of suitable polymer precursors are those represented by the generic formula



in which R^1 and R^2, which may be the same or different, represent optionally substituted lower alkyl groups, ie alkyl groups of 1 to 6 carbon atoms. In one preferred case, at least one, and preferably both, of R^1 and R^2 represents methyl.

Because the method of the invention involves the use of a number of different materials (the solvent, eg acetone, the precipitant, eg methanol, and water), each of which may be a good solvent for a particular bioactive material, the invention provides a number of different routes by which such a bioactive material may be incorporated into the polymer matrix. The bioactive material may be dissolved, for example, with the polymer in the solvent, or in the precipitant, or in water used for washing the precipitated matrix.

Mixtures of the above-defined polymers may be used, as may copolymers.

The method of the invention allows incorporation of a wide range of materials in the matrix including natural and synthetic polymers, particulates and powders of bioceramics for variation of material properties and for modulating the release of incorporated bioactive molecules.

Other materials may be therefore added to the primary solution in step a) to vary certain properties of the finished material such as degradation rate, density, thermal, mechanical, morphological and chemical characteristics.

The method of the invention is potentially useful for blending one or more different polymers (synthetic or natural) with PCL to obtain different physico-chemical characteristics so as to control the pattern of drug release from the blended polymer matrix or to modulate cell interaction with the material. The degradation rate of the poly (α-hydroxy acids) such as PLA and PLG can be varied from several weeks to over a year by copolymerisation, control of molecular weight, crystallinity and morphology. Blending of such polymers with PCL using the method of the invention would allow control of the degradation characteristics of the blend.

Certain non-water soluble, synthetic polymers may be co-dissolved with PCL in step a) to produce a blended solution. Hardening of the material in step b) may subsequently produce a microporous polymer blend.

Useful polymers include the poly α-hydroxy acids (PLA, PLG, DL.PLA), polymers of lactones, copolymers produced from lactide and non-lactide monomers such as lactones (eg ε-caprolactone) or ethylene glycol, PMMA, PU, copolymers containing a thermoplastic elastomer or hydrogel-forming copolymers such as poly(hydroxyethyl methacrylate).

PCL may be blended with polyethylene glycol (PEG), polyethylene oxide (PEO), copolymers of poly(ethyleneoxide)-poly(propylene oxide) (eg Poloxamer, Pluronic, Tetronic copolymers), or polyvinylpyrrolidone (PVP) in step a) to prepare microporous materials having a water soluble phase. Such compositions may be useful for controlling the rate and time of drug release from the polymer matrix.

The method of the invention is useful for incorporating various particulate fillers in the microporous PCL matrix to produce composite materials. Particulates may be mixed or homogenised with the solution in step a) prior to moulding.

Adjustment of particle size and density along with the viscosity of the solution allows control over particle sedimentation rate and subsequently the dispersion of the filler material.

Examples of useful particulate materials include natural materials such as polysaccharides (inulin, starch, dextran, cellulose and derivatives), sugar spheres, spray dried therapeutic polypeptides such as growth factors, proteins such as vaccine antigens and decalcified freeze dried bone (DFDB).

Other particulate fillers include bioceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) which have been widely investigated for production of bone substitutes and implant coatings, carbon, calcium carbonate, bioactive ceramic materials such as that known as 'Bioglass', etc

Examples of particulate synthetic polymers include PMMA powders such as those used in bone cements for implant fixation, polyesters (such as polyethylene polybutylene terephthalate, polyamides, PUS), biodegradable polymers such as

PLA and PLG, polyorthoesters, polyanhydrides, oligosaccharide ester derivatives (OEDs) etc.

Discontinuous 'chopped' fibre fillers could include alumina, carbon and synthetic polymers such as polyester 'Dacron', polyamides, PCU and its co-polymers POLA, PGA, polydioxanone (PDS), PU etc.

In one embodiment the invention provides block-form material which may be machined to the required form for implantation, eg bone graft substitutes.

Alternatively, the solution in step a) may be formed or poured into a shaped mould which replicates the shape of the tissue to be repaired (eg craniofacial bone segments). This reduces or avoids the need for final shaping of the implant. The method also allows for inserts to be 'moulded in' to provide attachment means to the host tissue eg suture sewing sites.

A layer or coating of microporous PCL may be applied to a device or encapsulation may be obtained by placing the component to be coated in the mould in step b).

Composite materials may be produced by impregnation of fibre preforms by PCL solution followed by hardening.

Since the precipitant may have undesirable toxicological or other properties, precipitation of the polymer matrix (step b)) will generally be followed by extraction of the precipitant, eg by washing with water. The matrix may also be dried after formation.

The release of incorporated bioactive factors from microporous PCL polymers will be influenced by the porous character (pore structure and connectivity) and material density of the delivery vehicle. These characteristics are adjustable by varying material composition, processing parameters such as the drying technique and by controlled compression of the drug-loaded material.

The microporous nature of the material results in increased surface area which can result in increased rates of degradation by hydrolysis and consequently provides an additional means of influencing drug release.

The microporous matrices of the invention are potentially useful in a wide range of tissue repair applications. They may be used as a binder for bioceramics, decalcified allogeneic bone and as a delivery system for growth factors in bone repair. The resilient characteristics of the microporous PCL material, coupled with high permeability may also find application in soft tissue reconstruction, eg cartilage repair.

The microporous materials may also be useful for fabricating controlled release rate delivery systems for bioactive molecules such as pharmaceuticals. Controlled release of contraceptive hormones or therapeutic polypeptide growth factors such as bone morphogenetic protein (BMP) is possible. Growth factors such as BMP may be incorporated in microporous materials by simple admixing of spray-dried particulates. In the case of therapeutic polypeptides, controlled delivery can overcome problems of short half lives and rapid absorption *in vivo* which can limit the efficacy of injected soluble formulations.

The rubbery characteristics of PCL result in high permeability which has been exploited previously for delivery of low molecular weight drugs such as steroids. The microporous materials produced in accordance with the invention may find application as 'depot-type' delivery systems for anti-cancer drugs such as leutenising hormone releasing hormone (LHRH) used to treat prostate cancer and for drugs such as Carmustine (BCNU) used in brain tumour therapy. The production of "matrix-type" transdermal delivery systems (TDDS) for nitroglycerin and transmucosal delivery systems for melatonine, low molecular weight heparin and proteins is also envisaged. Controlled release of DNA, oligonucleotides or vaccine antigens such as tetanus toxoid from depot-type devices is a further possibility.

Routes of drug administration for depot-type delivery devices formulated from microporous materials of the invention could include occular delivery, sub-cutaneous, intramuscular, intra-brain implantation, transdermal, and transmucosal routes.

Controlled release of agrochemicals is another potential field of application.

The invention will now be described in greater detail, by way of illustration only, with reference to the following Examples.

In all the Examples poly(ε-caprolactone) (molecular weight (MW) 50,000 by reduced viscosity measurements, Solvay-Interox) was used unless otherwise stated.

Example 1

1. A 12.5% w/v solution of PCL in acetone (4ml) was prepared by warming to approximately 50°C.

2. The solution was poured into a 10ml polypropylene (PP) mould and allowed to stand at room temperature for 30 minutes.

3. Methanol (6ml) was introduced carefully into the mould to form a layer on top of the PCL solution.

Rapid precipitation of the polymer was apparent at the interface between the PCL solution and methanol phase forming a film. The interfacial film serves to stabilise the interface and provides a semi-permeable membrane for exchange of solvent and non-solvent between each phase.

4. After 24 hours at room temperature, the sample had hardened by precipitation of the PCL polymer due to acetone extraction and could be removed from the PP mould.

5. The material was immersed in water (40ml) for 3 days to extract the methanol non-solvent with a change in medium every 24 hours.

6. Samples were dried in air at room temperature resulting in a white, uniform cylindrical material with an absence of voids and cracking (visual observation).

Examination of the PCL material by Scanning Electron Microscospy (SEM) revealed a surface comprising flat smooth areas separated by 100µm width striations which revealed the underlying microporous nature of the material. The material morphology was produced by a fibrous, lamellar structure giving rise to irregularly shaped pores generally of the order of 1-10µm in dimension.

The density of PCL microporous material determined by weighing 2 mm thick discs cut from cylindrical mouldings was $0.29\pm0.002 g/cm^3$.

Example 2

1. A 12.5% w /v solution of PCL in acetone (4ml) was prepared by warming to approximately 50°C.

2. The solution was poured into a 10ml PP mould and allowed to stand at room temperature for 30 minutes.

3. Methanol (6ml) was introduced into the mould to form a layer on top of the PCL solution.

4. The sample was retained at room temperature for 24 hours to produce hardening of the PCL polymer by precipitation.

5. The material was demoulded and immersed in water (50ml) for 7 days to extract the methanol with a change in medium every 24 hours.

6. Samples were dried in air at room temperature. The sample shrinkage was measured periodically and the results are presented in Table 1 for two cylindrical blocks of material prepared as described above. Shrinkage values were obtained using 1mm thick specimens cut from each block after Stage 5.

Table 1 Drying-induced shrinkage of microporous PCL material

Sample A		Sample B	
Drying time (days)	Diametral Shrinkage (%)	Drying time (days)	Diametral Shrinkage (%)
0	0	0	0
1	7.7	1	8.3
4	11.5	2	12.5
6	11.5	5	12.5
7	11.5	8	12.5
12	11.5		

It can be seen that sample shrinkage stabilised after 2-4 days drying at room temperature.

Example 3

1. A 12.5% w/v solution of PCL in acetone (4ml) was prepared by warming to approximately 50°C.

2. The solution was poured into a 10 ml PP mould and allowed to stand at room temperature for 1 week.

3. Methanol (6ml) was added to the mould to form a layer on top of the PCL solution.

4. The sample was retained at room temperature for 16.5 hours to produce hardening of the PCL polymer by precipitation.

5. The material was demoulded and immersed in water (40ml) for 0 and 1 day respectively, to extract the methanol non-solvent.

6. Samples were dried in air at room temperature. The sample shrinkage was measured periodically using 1.0 to 3.0 mm thick specimens cut from cylindrical blocks after 0 and 1 day immersion respectively in water (Stage 5). The diametral shrinkage of disk-shaped samples was measured at the times indicated in Table 2.

Table 2 Effect of water immersion on shrinkage of PCL microporous materials during drying.

Immersion time in water			
0 Days		1 Day	
Drying time (hours)	Shrinkage (%)	Drying time (hours)	Shrinkage (%)
0	0	0	0
1	14.3	–	–
3.5	14.3	–	–
6.5	14.3	–	–
24	14.3	24	7.7
48	10.7	–	–
144	–	144	11.5
168	10.7	–	–

Samples dried immediately after precipitation with methanol (Stage 4) exhibited shrinkage values after 6 days air drying which were similar to samples immersed in water for 1 and 7 days respectively prior to drying (Tables 1 and 2). The former samples tended, however, to exhibit a coarser microstructure or texture than water treated materials, illustrating the potential for controlling the material morphology by varying the drying process.

The initial shrinkage of samples which were precipitated using methanol but not immersed in water, was higher at 24 hours than at 48 hours (Table 2). This behaviour is probably explained by a plasticising effect of non-solvent molecules initially which facilitates chain recoiling and by recovery/ relaxation processes subsequently within the constrained macromolecular structure of the polymer.

Example 4

Production of particulate-filled PCL materials

Microporous hydroxyapatite/PCL material

1. Sub-micron, hydroxyapatite (HA) powder (PlasmaBiotal) (500mg) was mixed with 1ml acetone to produce a slurry. This was dispersed in 4ml PCL solution in acetone (12.5% w/v) and added to a 10ml PP mould.

2. Methanol (5ml) was added, forming a layer on top of the suspension and the sample was retained at room temperature for 4 days to allow complete solidification of the PCL matrix.

3. On demould, the material was immersed in water for 3 days with a change of medium every 24 hours.

4. The sample was air dried at room temperature to produce a hard, white material.

SEM examination of the HA/PCL composites revealed a dispersion of HA particulates within a microporous polymer matrix.

The density of HA/PCL composite material, determined by weighing 2mm thick discs cut from a cylindrical mouldings was 0.49g/cm^3.

Example 5

Production of particulate-filled PCL materials.

Microporous Inulin/PCL materials

1. Inulin powder (1gm, from Chicory Root, Sigma Chemicals) was mixed with 4ml 12.5% w/v PCL solution in acetone and the suspension was poured into a 10ml PP mould.

2. Methanol (5ml) was added and the sample was retained at room temperature for 4 days to allow precipitation and hardening of the PCL matrix.

3. On demould, the sample was immersed in 40ml water for 3 days with a change of medium every 24 hours.

4. Samples were dried in air at room temperature producing a hard, white material.

The density of the inulin/PCL composite material, determined by weighing 3mm thick discs cut from cylindrical mouldings was 0.89±0.002g/cm^3.

Example 6

Production of particulate-filled PCL materials.

Microporous PMMA/PCL materials

1. PMMA bone cement powder (500mg, DePuy-CMW) was mixed with 4ml 12.5% w/v PCL solution in acetone and the suspension was poured into a 10ml PP mould.

2. Methanol (5ml) was added and the sample was retained at room temperature for 1 day to allow hardening to occur.

3. On demould, the sample was immersed in 40ml water for 4 days with a change of medium every 24 hours.

4. Samples were dried in air at room temperature producing a hard, white material.

The density of PMMA/PCL composite material, determined by weighing a 12.0mm diameter x 11mm cylinder was 0.33g/cm^3.

Example 7

Weight loss characteristics

Samples of PCL microporous material produced as described in Example 2 and inulin/PCL materials produced as described in Example 5 were immersed in PBS at 37°C for time periods up to one year. The weight loss figures are presented in Table 3.

Table 3

Time in PBS (months)	Weight loss (%w/w)	
	Microporous PCL	Inulin/PCL
1	0	10.2
2		10.7
4		13.9
9		17.6
12	0	17.6

This shows that resorption characteristics can be modified by incorporation of other materials in the microporous PCL matrix.

Example 8

Cell culture experiments

Primary human osteoblast cells (HOB) were seeded onto samples of PCL microporous materials, HA-filled PCL materials and Thermanox controls contained in 48-well plates at a density of 50,000 cells per well. At time points of 90 minutes, 4 and 24 hours the samples were removed and processed for examination of cell

morphology by environmental scanning electron microscospy (ESEM). The results are presented in Table 4.

Table 4

Culture time point	Appearance of Cells	
	PCL microp. - HA/PCL	Thermanox
90 minutes	rounded, cell processes visible	rounded and spread cells
4 hours	spread, forming cell layer	spread
24 hours	uniform cell layer, individual cells not distinguishable	spread, individual cells discernible

The observation of cell spreading on PCL microporous materials to form uniform layers of coalesced cells indicates production of a highly favourable surface for bone cell interaction and growth.

Claims

1. A method for the manufacture of a microporous polymer matrix, which method comprises the steps of

a) introducing into a mould a solution of polymer in a solvent, and

b) adding a precipitant to the solution so as to cause precipitation of the polymer from the solution in a form which retains the shape of the mould upon removal from the mould.

2. A method as claimed in Claim 1, wherein the solvent is an organic solvent.

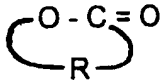
3. A method as claimed in Claim 2, wherein the solvent is acetone or ethyl acetate.

4. A method as claimed in any preceding claim, wherein the precipitant is a solvent in which the polymer is substantially insoluble.

5. A method as claimed in Claim 4, wherein the precipitant is an alcohol.

6. A method as claimed in any preceding claim, wherein the solution of polymer and the precipitant form distinct layers when the precipitant is brought into contact with the solution of polymer.

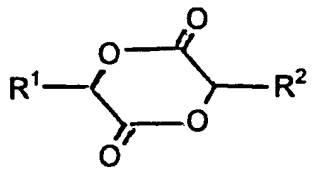
7. A method as claimed in any preceding claim, wherein the polymer is a polyester formed by ring-opening polymerisation of a precursor of the generic formula



in which R represents an optionally substituted alkylene chain, eg a chain $(CH_2)_n$ in which n is an integer of from about 4 to 10.

8. A method as claimed in Claim 7, in which the polymer is poly(ε-caprolactone).

9. A method as claimed in any one of claims 1 to 6, in which the polymer is a polymer of a precursor of the generic formula



in which R^1 and R^2, which may be the same or different, represent optionally substituted lower alkyl groups, ie alkyl groups of 1 to 6 carbon atoms.

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/GB 00/04472

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08J9/28 A61L27/18 //C08L67/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08J A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 290 494 A (COOMBES ALLAN G A ET AL) 1 March 1994 (1994-03-01) cited in the application	1-5,7-9
A	examples 1,15 claims	6
X	FR 2 144 102 A (POLAROID CORP) 9 February 1973 (1973-02-09)	1,2,4,5
A	example 1 claims	6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2001	27/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oudot, R

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/GB 00/04472

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5290494 A	01-03-1994	US 5397572 A	14-03-1995
		US 5492697 A	20-02-1996
FR 2144102 A	09-02-1973	NONE	